Tobacco Plants as Biofactories: Rapid and Cost-Effective Production of an Anthrax Therapy

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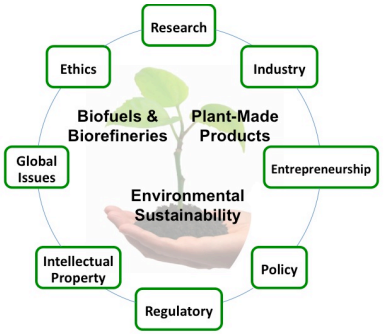
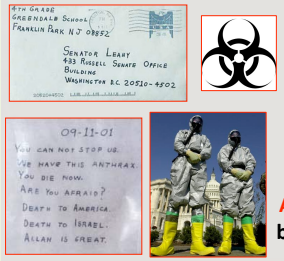
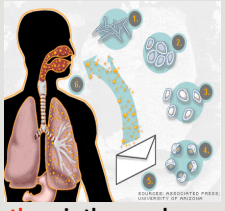


Interdisciplinary teams of graduate students and faculty trainers use **plants**, **biotechnology**, and **engineering** to help solve societal problems, including global health, energy independence, and agricultural sustainability.

Bioterrorism: The Anthrax Scare of 2001





Anthrax is the number one biological warfare threat to the United States.

Motivation: Using Plants for Rapid Response Manufacturing of Therapeutics In Case Of Outbreaks

Problem: Current production methods for therapeutics are unable to supply the demands created by large-scale outbreaks, such as **H1N1** or **anthrax**.

Solution: Plants can be used as a manufacturing platform for **therapeutic proteins.** The advantages are:

- **Speed of production** – large-scale production in weeks instead of months
- **Cost-effectiveness** – manufacturing costs are 5% of the current methods
- **Safety** – plants do not propagate human viruses or pathogens
- **Product quality** – capable of protein post-translational modifications
- **Non-transgenic** – process is contained, regulatory hurdles are reduced

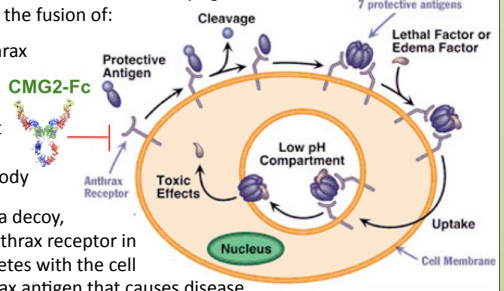
The Manufacturing Platform: Tobacco Plants

- **Easily transformable** – gene of interest can be easily delivered into the plant
- **Non-food, non-feed crop** – no issues about using crops that divert from the food supply
- **Economical productivity** – several acres are sufficient to produce millions of vaccine doses over the course of a year

The Therapy: CMG2-Fc, A New Antibody Against Anthrax

CMG2-Fc is an engineered human antibody against anthrax. It is composed of the fusion of:

- **CMG2** – the anthrax cell receptor in humans
- **Fc** – the constant fragment of the human IgG antibody

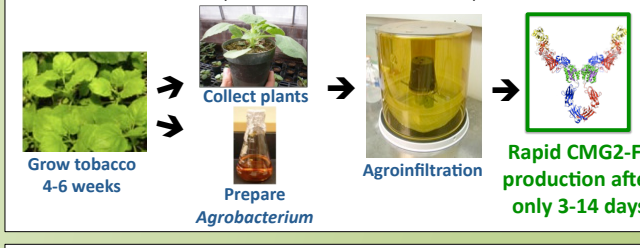


CMG2-Fc works as a decoy, mimicking the anthrax receptor in humans. It competes with the cell to bind the anthrax antigen that causes disease.

CMG2-Fc acts as a regular antibody, and causes its therapeutic effect by inhibiting the production of the lethal anthrax toxins.

Agroinfiltration: CMG2-Fc DNA Can Be Easily Transferred to the Tobacco Plant

Agrobacterium is nature's genetic engineer, because it naturally transfers genetic material into plants. We are able to engineer *Agrobacterium* to transfer the DNA of the protein of interest we want to produce.



My Research Project: Using Gene Silencing Suppressors to Increase Yield of the CMG2-Fc Antibody in Tobacco

- **Gene silencing** is a natural defense system of plants against viruses and pathogens. It is also activated when a foreign protein is produced in the plant.
- **Gene silencing suppressors** are a class of proteins obtained from plant viruses that are able to suppress the gene silencing mechanism in plants.
- **Hypothesis**: the use of gene silencing suppressors can lead to increased yields of the CMG2-Fc anthrax antibody in tobacco plants.

Objective

Test **9 different gene silencing suppressors** to determine their impact on the amount of CMG2-Fc protein produced in tobacco plants.

Experimental Design

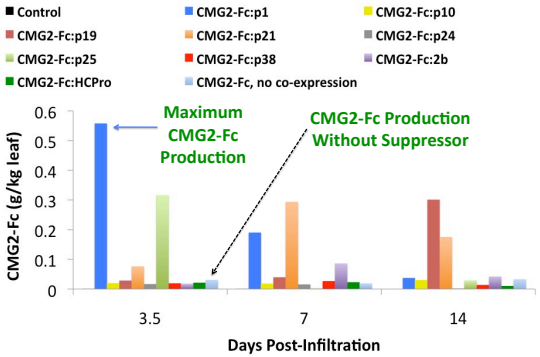
Three tobacco plants were agroinfiltrated with 2 strains of *Agrobacterium* corresponding to each of the following co-production conditions:

- CMG2-Fc and **p1** suppressor
- CMG2-Fc and **p10** suppressor
- CMG2-Fc and **p19** suppressor
- CMG2-Fc and **p21** suppressor
- CMG2-Fc and **p24** suppressor
- CMG2-Fc and **p25** suppressor
- CMG2-Fc and **p38** suppressor
- CMG2-Fc and **HCPro** suppressor
- CMG2-Fc and **p2b** suppressor
- **CMG2-Fc** without suppressor

Methods and Results

ELISA - assay used for specific quantification of the CMG2-Fc protein

CMG2-Fc Production With Different Gene Silencing Suppressors



Conclusions

- Certain gene silencing suppressors – **p1**, **p19**, **p21**, and **p25** – increased antibody production in tobacco, while the others seemed to have no effect on the yield, as measured by ELISA
- The highest level of CMG2-Fc was produced after just 3.5 days with the **p1 gene silencing suppressor**, at 0.56 g/kg of leaf**, a 10 times increase** when compared to absence of suppressor.
- Further optimization of the yields obtained with this platform (to levels above 1 g/kg) will be important in developing this manufacturing technology for commercial use.

Acknowledgements

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